

## Evaluation of agromorphological and grain physical traits in Greek barley accessions

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**Abstract.** This study assessed the agromorphological and grain physical traits of twelve barley (*Hordeum vulgare* L.) accessions, comprising eight Greek landraces and four cultivars, over two consecutive growing seasons (2022–2024). Significant genotypic effects and accession-by-year interactions were observed for most agromorphological traits, particularly plant height, spike morphology, and grain yield components. Grain physical characteristics, evaluated during one season, also revealed clear genotypic differentiation. Specific landraces, H1 (from Amorgos Island), H8 (from Pyrgos Region), and the old cultivar Athinaida, demonstrated high productivity and adaptability, performing comparable to, or even surpassing, modern cultivars under variable Mediterranean conditions. Accessions originating from the Greek islands produced smaller but firmer grains, traits that likely reflect adaptation to arid environments, whereas those from the mainland and modern cultivars exhibited larger, less firm grains. Principal Component Analysis (PCA) and cluster analysis, integrating both agromorphological and grain physical traits, revealed consistent patterns of differentiation, grouping accessions according to their geographic origin and breeding status. These findings highlight the genetic value and adaptive potential of Greek barley landrace genetic material for breeding programs aiming to improve resilience, quality, and yield stability in low-input agricultural systems.

**Key words:** genotype-environment interaction, phenotypic variability, grain morphology, traditional germplasm, yield components.

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops globally, particularly in the Mediterranean region, where it has been cultivated for thousands of years. As one of the first crops to be domesticated in the Near East Fertile Crescent (Harlan & Zohary, 1966), barley (*Hordeum vulgare* ssp. *vulgare*) is considered an excellent model for understanding how major crops adapt to climate change (Dawson et al., 2015; Zhou et al., 2024).

In Greece, recognized as an important center of barley evolution and diversification (Jakob et al., 2014), barley plays a significant role in human nutrition, livestock feeding, and the brewing industry. Barley landraces, also referred to as traditional, local, or farmers varieties, or local populations have evolved over centuries of traditional farming practices and under the specific environmental conditions of the Mediterranean (Ceccarelli et al., 1998). They are genetically heterogeneous populations that have been developed through natural and human selection in specific eco-geographical regions (Casañas et al., 2017; Lazaridi et al., 2024) and exhibit high levels of genetic diversity (Brbaklić et al., 2021). This diversity provides resilience against multiple biotic and abiotic stresses, such as drought, low soil fertility, and pathogen infections (Škipars et al., 2021; Vasilaki et al., 2023).

Nevertheless, the intensification of agriculture has led to genetic erosion and the loss of valuable traits critical for long-term agricultural sustainability (Ficiciyan et al., 2018; Robbana et al., 2023). Despite their agronomic and ecological value, many barley landraces have been gradually replaced by commercial cultivars bred for uniformity and high productivity under intensive conditions (Gadissa et al., 2021). In Greece, this trend is particularly evident, with traditional varieties now occupying only a small fraction of cereal cultivation, mostly in remote areas (Thanopoulos et al., 2021). This loss of biodiversity causes genetic erosion and undermines efforts toward sustainable agriculture. Barley landraces, shaped by long-term adaptation, possess traits such as yield stability, efficient resource use, and stress tolerance, making them valuable genetic materials for cultivation in low-input environments and for incorporation into modern breeding programs (Monteagudo et al., 2019; Kumar et al., 2020).

Recent studies underscore the importance of evaluating key agromorphological and grain physical traits in landraces, as these characteristics influence yield potential, end-use quality, and processing performance (Baik & Ullrich, 2008; Assadzadeh et al., 2022; Ghazy et al., 2024). A better understanding of phenotypic diversity can reveal genotypes with improved performance under environmental stresses, contributing to resilient farming systems (Visioni et al., 2023). Systematic classification and phenotypic evaluation methods, like those used in other cereals, provide insights into the agronomic potential of local varieties (Protonotariou et al., 2023; Spanic et al., 2024). These efforts support the identification of promising genotypes for breeding and the development of conservation strategies to safeguard this valuable genetic and cultural heritage.

This study aims to assess the plant agromorphological and grain physical traits of selected Greek barley landraces. By identifying key parameters that contribute to yield, resilience, and quality, it seeks to provide essential information for their conservation and strategic use in modern agriculture, with broader implications for biodiversity and food security.

## MATERIALS AND METHODS

### Plant material

The plant material comprised twelve *Hordeum vulgare* L. accessions, including three commercial cultivars, one old cultivar, and eight landraces (Table 1). The landraces were collected from geographically and ecologically isolated regions across both insular and mainland Greece (Fig. 1), aiming to capture the country's agroecological diversity.

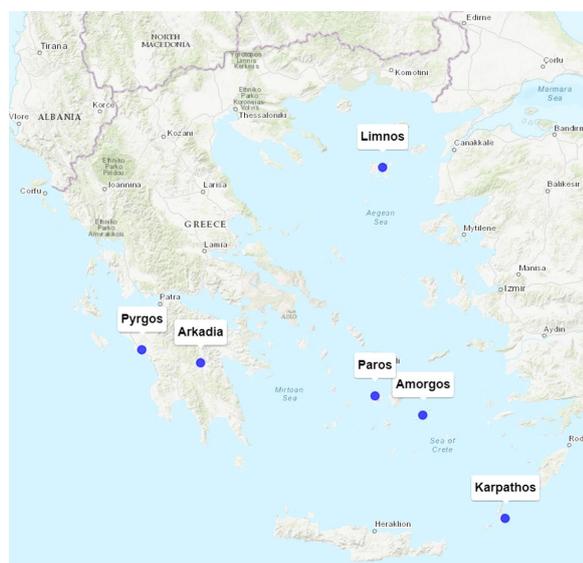
**Table 1.** Origin, breeding status, and geographical coordinates of the barley accessions evaluated in the study

Code	Accession name	Breeding status	Location of origin
H1	-	Landrace	Amorgos, Cyclades, Aegean
H2	-	Landrace	Amorgos, Cyclades, Aegean
H3	-	Landrace	Arkadia, Peloponnese
H4	-	Landrace	Karpathos, Dodecanese, Aegean
H5	-	Landrace	Panagia, Limnos, North Aegean
H6	-	Landrace	Dafni, Limnos, North Aegean
H7	-	Landrace	Paros, Cyclades, Aegean
H8	-	Landrace	Prasino, Pyrgos, Peloponnese
H9	Athinaida	Old cultivar (selection from landrace population)	-
H10	Triptolemos	Cultivar	-
H11	Thessaloniki	Cultivar	-
H12	Explora-R2	Cultivar	-

The old cultivar, Athinaida, originated from a selection within a local insular landrace population (Mylonas et al., 2014). All landraces were donated to the Plant Breeding and Biometry Laboratory of the Agricultural University of Athens (AUA) for further evaluation and conservation. The commercial cultivars were provided by the Institute of Plant Breeding and Genetic Resources - Thessaloniki, Greece (IPBGR) and the company Agroten SA Greece. Among the twelve accessions, eleven were classified as six-row barley and one (Thessaloniki) as two-row. All accessions used in this study were hulled barley, a type typical of barley cultivated in Greece.

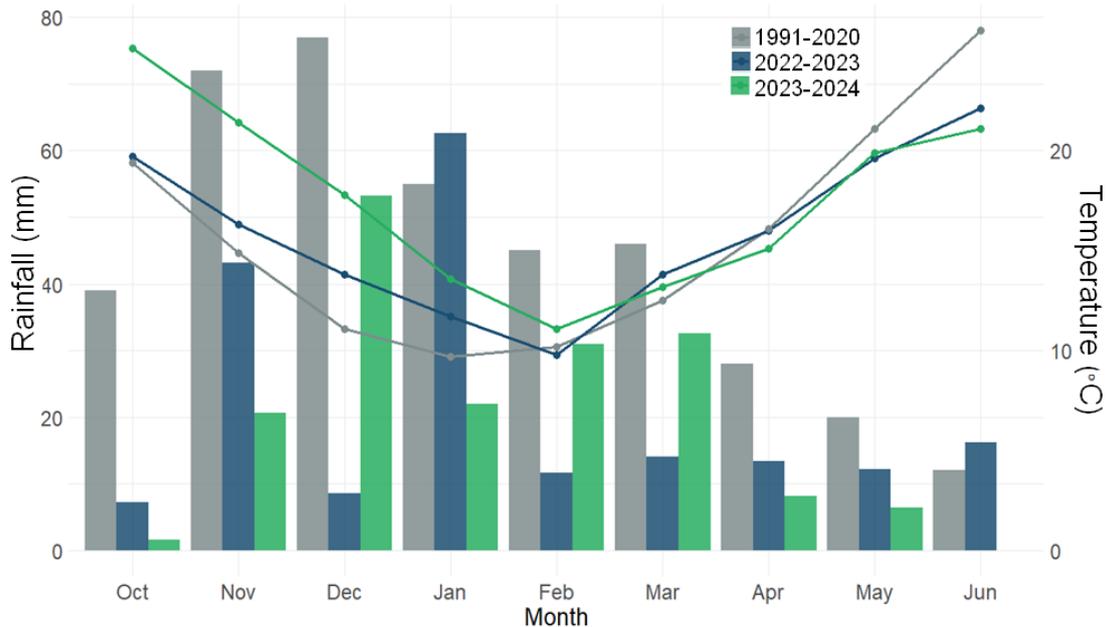
### Experimental design

The field trials were conducted over two consecutive growing seasons (2022–2024) at the experimental field of the Agricultural University of Athens (37°59'06.8" N, 23°42'24.7" E; altitude 24 m). A randomized complete block design (RCBD) with three replications was used. Each complete block included all twelve barley accessions, resulting in 36 experimental plots. Each plot consisted of four rows (1 m long, 0.25 m row spacing, 0.10 m between plants). Sowing took place in mid-November and harvest at the end of May. Prior to sowing, basic tillage was performed, which included plowing and harrowing to prepare the soil. Basic fertilization was applied according to the nutritional needs of the plants, using a mix of macro- and micronutrients based on



**Figure 1.** Geographical distribution of the barley accessions collected across mainland and insular Greece.

soil analyses. Weed control was mechanical, while irrigation and plant protection treatments were applied only, when necessary, mainly during flowering and grain filling. The soil was classified as clay loam with neutral pH and moderate organic matter. Meteorological data for the two growing seasons (mean temperature and total precipitation), as well as the average values for the last 30 years (1991–2020), were obtained from the National Observatory of Athens (NOA) and are presented in Fig. 2.



**Figure 2.** Monthly distribution of total rainfall and mean air temperature during the barley growing seasons of 2022–23 and 2023–24, and the long-term climate means for the period 1991–2020.

### Plant agromorphological and grain physical trait measurements

A comprehensive set of agromorphological and grain physical traits was evaluated to characterize plant performance and grain physical properties (Table 2). Among the physical traits, the geometric (e.g., diameter, perimeter, area) and colorimetric characteristics ( $L$ ,  $a^*$ ,  $b^*$ , whiteness index) were measured on dehulled grains. Thousand kernel weight (TKW) was determined by weighing 1,000 randomly selected kernels, subsequently used to calculate bulk and tap densities. Bulk density ( $\rho_b$ ) was defined as grain mass per unit bulk volume, whereas tap density ( $\rho_t$ ) was measured after applying 100 mechanical taps to reduce void spaces. The Carr Index (CI), calculated as  $CI (\%) = [(\rho_t - \rho_b) / \rho_t] \cdot 100$ , was used to assess flowability, an important parameter for processing and storage.

Grain firmness was measured using an Instron Universal Testing Machine (Model 3343, Nordwood, MA, USA) equipped with a 1 kN load cell. Fifty intact grains per accession were compressed to 50% of their original width using a 4 cm diameter probe at a speed of  $1 \text{ mm min}^{-1}$ . Grain color was evaluated with a spectrophotometer (NS800S, Shenzhen 3nh Technology, China) under the CIE-Lab\* color space, recording  $L^*$  (lightness),  $a^*$  (green–red spectrum), and  $b^*$  (blue–yellow spectrum) values. The whiteness index (WI) was also calculated to capture variations in grain appearance.

Grain shape and size parameters were analyzed through image processing. One hundred grains per accession were scanned twice using an HP Scanjet 4370 (Hewlett-Packard, USA), and processed with Image Pro Plus 7 software (Media Cybernetics, USA). Geometric traits included grain area (mm<sup>2</sup>), aspect ratio, average optical density, minimum and maximum diameters (mm), perimeter (mm), roundness, and overall grain dimensions (length and width in mm). All measurements were performed in triplicate to ensure reproducibility and statistical accuracy.

**Table 2.** Description of Measured Agromorphological and Grain Physical Traits

Trait name (Abbreviation)	Unit	Methodology
Plant height	cm	Measured from soil surface to the tip of the spike
Plant weight	g	Dry weight of total aboveground plant biomass
Number of stems	no.	Number of all stems per plant
Number of spikes	no.	Number of spikes per individual plant
Spike length	cm	Measured from spike base to tip, excluding awns
Number of spikelets	no.	Total number of spikelets per main spike
Weight of main spike	g	Dry weight of the main spike per plant
Weight of main spike grains	g	Dry weight of grains from the main spike
Weight of other spikes' grains	g	Dry weight of grains from the secondary spikes
Number of grains	no.	Total number of grains per plant
Total yield	g	Combined dry grain weight per plant
Thousand kernel weight (TKW)	g	Weight of 1000 grains
Volume	cm <sup>3</sup>	Measured grain volume
Tap volume	cm <sup>3</sup>	Grain volume after tapping
Bulk density	g cm <sup>-3</sup>	Grain mass per unit volume (without compaction)
Tap density	g cm <sup>-3</sup>	Grain density after 100 mechanical taps
Carr Index (CI)	%	Calculated as $[(\rho_t - \rho_b) / \rho_t] \cdot 100$
Grain firmness (SF)	N (Newton)	Force required to compress grain to 50% width
Grain color (lightness) (L*)	CIE-Lab*	L = seed lightness (spectrophotometer)
Grain color (green-red) (a*)	CIE-Lab*	a = green (-) to red (+) (spectrophotometer)
Grain color (blue-yellow) (b*)	CIE-Lab*	b = blue (-) to yellow (+) (spectrophotometer)
Whiteness Index	Index	Calculated index reflecting grain surface whiteness
Seed area (Area)	mm <sup>2</sup>	2D projected area of grain (image analysis)
Aspect ratio	Ratio	Ratio of grain length to width
Average density (Density)	Unitless	Mean grain density
Diameter max	mm	Shortest cross-sectional width from image analysis
Diameter min	mm	Longest cross-sectional length from image analysis
Diameter ave	mm	Mean cross-sectional diameter from image analysis
Perimeter	mm	Total boundary length of grain from image analysis
Roundness	Unitless	Shape index ( $4\pi \cdot \text{area}/\text{perimeter}^2$ ); higher = rounder
Grain length	mm	Longest seed axis (image analysis)
Grain width	mm	Width perpendicular to length (image analysis)

### Statistical analysis

Statistical analysis was performed using R (R Core Team, 2024). All agromorphological and grain physical traits were measured at the individual plant level. The quantitative variables were subjected to analysis of variance (ANOVA) after verifying the assumptions of normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively (Fox & Weisberg, 2019). Post hoc

comparisons among means were conducted using Tukey's Honest Significant Difference (HSD) test at a significance level of  $\alpha = 0.05$  (de Mendiburu, 2023). Accession  $\times$  year interaction analysis was applied only to agromorphological traits, as grain physical traits were evaluated during a single growing season. Pearson's correlation analysis was conducted using accession means. Principal Component Analysis (PCA) was performed to reduce the dimensionality of the agromorphological and grain physical traits, allowing for a clearer visualization of the relationships among the accessions. This analysis helped to identify key patterns in the data and revealed the main sources of variability within the dataset. Cluster analysis was performed on standardized data using hierarchical clustering (Maechler et al., 2025), with the Ward linkage method and Euclidean distance as the dissimilarity metric.

## RESULTS AND DISCUSSION

### Plant Agromorphological Traits

Mean values of quantitative agromorphological traits for all studied barley accessions are summarized in Table 3. A highly significant genotypic effect was observed across most traits, particularly for plant height, spike morphology, grain number, and total yield. Additionally, year-to-year variation had a significant effect, underscoring the influence of environmental fluctuations between growing seasons. The 2022–2023 season, characterized by more moderate climatic conditions, was associated with enhanced biomass accumulation and increased grain weight. In contrast, the 2023–2024 season, which experienced generally warmer temperatures and lower rainfall (particularly in June), resulted in taller plants and longer spikes, but generally lower grain yields (Bento et al., 2021). This distinction highlights the complex interaction between climatic factors and plant development, where warmer temperatures in the 2023–2024 season, coupled with reduced rainfall, may have led to increased vegetative growth but reduced reproductive success, affecting overall yield (Hossain et al., 2012).

Plant height varied significantly among accessions, with landraces H1 and old barley cultivar Athinaida (H9) producing the tallest plants ( $> 78$  cm), while cultivar Triptolemos exhibited the shortest stature (63.26 cm). This height difference could be attributed to traditional selection for biomass and competitive ability in landrace genetic material, as well as to the absence of dwarfing genes that are commonly introduced in modern cultivars (Richards et al., 2002). Similarly, previous studies have highlighted the genetic diversity of landraces in terms of plant stature, with crop landraces generally showing greater height compared to modern cultivars (Radic et al., 2024). Interestingly, the strong positive correlation between plant height and spike length did not translate into higher productivity, suggesting resource allocation trade-offs typical of Mediterranean environments (Richards et al., 2002; Brunner et al., 2024). These findings are in line with other research that has indicated that, while taller plants may develop longer spikes, they do not necessarily result in higher yields (Mullualem et al., 2024; Hlisenikovsky et al., 2024).

**Table 3.** Mean values of quantitative agromorphological traits and multiple comparisons among barley accessions using Tukey's *test* ( $\alpha = 0.05$ )

Treatments	Plant height (cm)	Plant weight (g)	Number of stems	Number of spikes	Spike length (cm)	Weight main spike (g)	Number of grains	Weight of main spike grains (g)	Weight of other spikes' grains (g)	TWK (g)	Total yield (g)
H1	80.59 <sup>a</sup>	24.49	9.51	7.47	11.96	1.61	40.13	1.32	3.87	32.68 <sup>ab</sup>	5.19
H2	76.49 <sup>abc</sup>	20.54	8.14	6.6	12.15	1.51	39.72	1.27	3.12	32.06 <sup>ab</sup>	4.35
H3	69.1 <sup>abc</sup>	17.68	7.78	3.92	11.09	4.7	38.56	1.01	1.24	25.51 <sup>bc</sup>	2.02
H4	70.45 <sup>abc</sup>	17.57	8.37	6.18	12.10	1.45	45.46	1.59	2.56	36.85 <sup>a</sup>	4.15
H5	66.29 <sup>bc</sup>	20.49	8.46	5.03	11.49	1.49	40.00	1.17	2.25	28.38 <sup>abc</sup>	3.62
H6	71.67 <sup>abc</sup>	25.34	8.36	6.08	12.82	4.10	41.55	1.12	2.63	26.77 <sup>bc</sup>	3.75
H7	74.98 <sup>abc</sup>	20.33	8.47	6.01	12.25	1.51	40.68	1.21	2.68	29.64 <sup>abc</sup>	3.59
H8	70.82 <sup>abc</sup>	30.21	9.07	7.02	12.26	1.62	40.26	1.37	3.84	33.01 <sup>ab</sup>	5.21
H9	78.02 <sup>ab</sup>	23.31	7.23	6.38	10.85	1.80	47.08	1.56	4.38	33.09 <sup>ab</sup>	5.93
H10	63.26 <sup>c</sup>	23.75	8.30	6.31	10.46	1.52	50.95	1.24	3.28	24.66 <sup>bc</sup>	4.50
H11	65.02 <sup>bc</sup>	18.60	7.70	6.75	13.77	2.95	26.10	0.84	2.25	32.19 <sup>ab</sup>	3.04
H12	67.04 <sup>bc</sup>	17.23	7.00	4.75	12.56	1.48	56.27	1.20	2.03	21.19 <sup>c</sup>	3.62
Prob > F <sup>1</sup>	*** <sup>1</sup>	ns	ns	***	***	ns	***	ns	***	*	***
2022–2023	53.94 <sup>b</sup>	27.00 <sup>a</sup>	9.39	7.01	6.21	2.71	43.33	1.45 <sup>a</sup>	3.31	33.76 <sup>a</sup>	4.69
2023–2024	88.35 <sup>a</sup>	16.25 <sup>b</sup>	7.01	5.07	17.75	1.58	41.13	1.04 <sup>b</sup>	2.38	25.58 <sup>b</sup>	3.50
Prob > F	***	***	***	***	***	*	*	***	***	***	***
Accession x Year	ns	ns	*	*	***	*	**	ns	***	ns	***
R <sup>22</sup>	0.92	0.57	0.57	0.71	0.99	0.49	0.80	0.55	0.78	0.56	0.80
CV% <sup>3</sup>	9.45	39.17	23.21	20.84	6.28	61.68	11.75	31.24	26.28	26.89	22.15
G. Mean <sup>4</sup>	71.14	21.63	8.20	6.04	11.98	2.14	42.23	1.24	2.84	29.67	4.09

<sup>1</sup> Prob > F: Significance level; \* / \*\* / \*\*\*:  $p < 0.05$  / 0.01 / 0.001; ns: Not significant: <sup>2</sup> R<sup>2</sup>: Coefficient of determination: <sup>3</sup> CV%: Coefficient of variation:

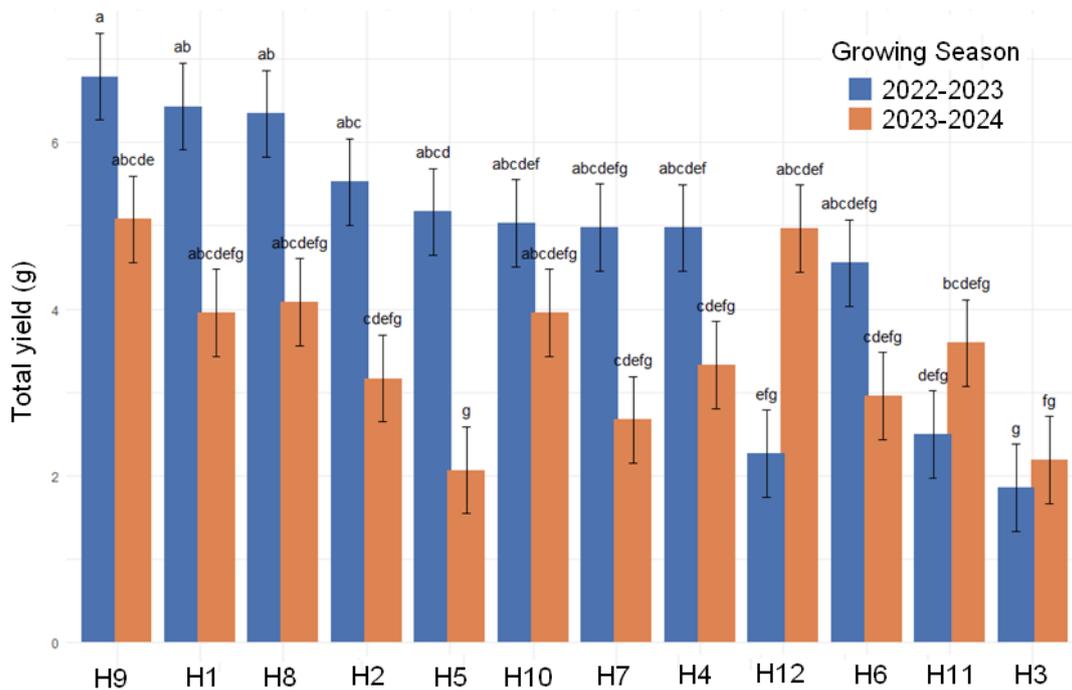
<sup>4</sup> G. Mean: Grand mean: Different letters indicate significant differences based on Tukey's *test*.

In terms of spike productivity, landrace H1 recorded the highest number of spikes per plant (7.47), closely followed by landrace H8 and old cultivar H9. H8 also exhibited the highest total plant biomass (30.21 g), indicating strong vegetative vigor. This vegetative vigor is a key factor in overall plant productivity, as confirmed by previous findings where higher biomass was positively correlated with greater yield potential (Křen et al., 2014). Main spike length and weight were significantly influenced by both genotype and growing season, with significant accession  $\times$  year interactions. The variability in spike characteristics due to environmental factors further supports the importance of genotype  $\times$  environment interactions in shaping crop performance (Hlisnikovský et al., 2024).

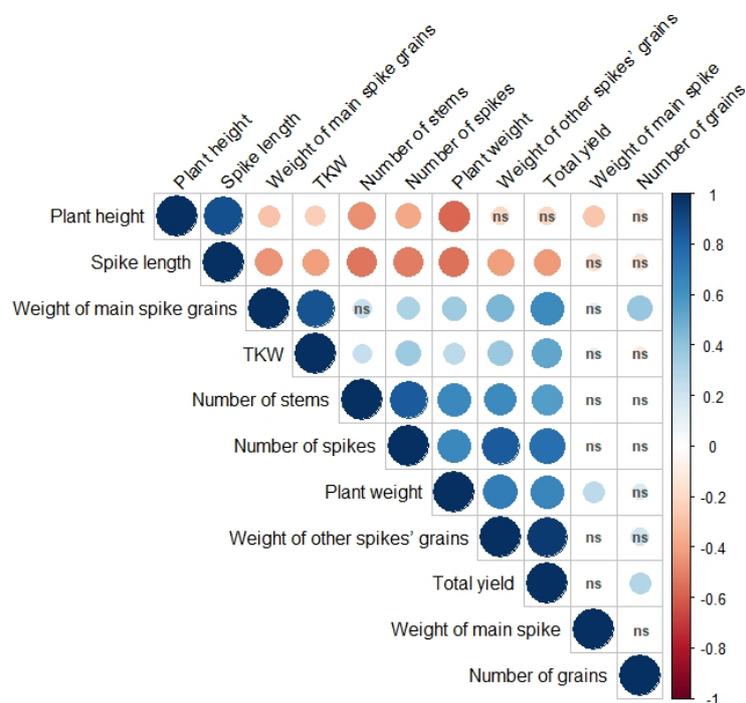
Yield-related traits further differentiated the accessions. While cultivar Explora-R2 produced the highest number of grains per plant (56.27), this did not translate into a high final yield due to relatively low grain weight. In contrast, landrace H8 and old cultivar H9 (*Athinaida*) achieved the highest grain yields (5.93 g and 5.21 g per plant, respectively) by combining high grain number with greater grain mass. This aligns with previous studies that highlight the importance of balancing both grain number and grain size for achieving high productivity (Zhang et al., 2021). Additionally, landrace H1 also demonstrated robust productivity. Conversely, landrace H3, despite producing the heaviest main spike (4.70 g), yielded poorly overall (2.02 g), likely due to a limited number of spikes and reduced grain set, further illustrating the complexity of yield formation.

Thousand-kernel weight (TKW), a key component of grain yield, showed significant variation among genotypes and across years. Landraces H4 (36.85 g) and old cultivar H9 (33.09 g) recorded the highest TKW values, indicating superior grain-filling capacity. The importance of TKW for overall yield potential has been well-documented, as higher TKW values are consistently associated with increased grain yield (Zhang et al., 2021). This trait plays a crucial role in determining final productivity and is a key target for breeders looking to improve barley yields.

The interaction between year and accession had a significant effect on barley yield. More specifically, the environmental conditions of the 2023–2024 growing season, characterized by warmer temperatures and lower rainfall compared to the 2022–2023 season and the long-term average (1991–2020), had a marked impact on the productivity of the barley accessions. Among the landraces, H7 exhibited the smallest yield decline (-32%), standing out for its superior adaptability compared to other landraces such as H8 (-36%) and H1 (-38%) (Fig. 3). This differential response to environmental stress is consistent with findings from other studies that emphasize the role of genetic adaptability in mitigating yield losses under adverse conditions (Hlisnikovský et al., 2024). Additionally, H5, although experiencing some yield reduction, maintained higher yields than many other landraces, suggesting potential resistance to environmental stressors, which is a valuable trait for sustainable agriculture. Notably, landrace H4, although not one of the top-performing germplasm, showed relative stability among the landraces, with a yield decline of only -25%, in contrast to the more significant reductions observed in others. This suggests that H4 may possess inherent traits that allow it to better withstand environmental stress (Salimi et al., 2023; Czembor & Czembor, 2025).



**Figure 3.** Multiple comparisons of total yield of barley accessions per Growing Season using the Tukey test. Different letters indicate significant differences ( $\alpha = 0.05$ ).



**Figure 4.** Correlation coefficients between quantitative agromorphological traits of the studied barley accessions ( $n = 72$ ). Circle size shows correlation strength; color indicates direction (red: negative, blue: positive). Non-significant correlations are denoted as 'ns'.

Pearson correlation analysis among the measured traits (Fig. 4) revealed several statistically significant associations. Total grain yield was significantly correlated with 1,000-kernel weight ( $r = 0.95$ ), grain weight from secondary spikes ( $r = 0.64$ ), and grain number from the secondary spikes ( $r = 0.65$ ). This reinforces the importance of both the main and secondary spikes in contributing to the final yield. The number of stems per plant was significantly correlated with both spike count ( $r = 0.83$ ) and spike length ( $r = 0.75$ ), suggesting that genotypes with higher tillering capacity also tend to produce longer and more numerous spikes. Additionally, spike length was significantly positively correlated with 1,000-kernel weight ( $r = 0.83$ ). These correlations support the additive value of both the main and secondary spikes in determining overall productivity (Radic et al., 2024). Plant height was significantly positively correlated with spike length ( $r = 0.87$ ), yet significantly negatively correlated with traits directly associated with seed yield, such as plant weight ( $r = -0.57$ ). This suggests that taller plants with longer spikes may not always achieve greater yield, likely due to biomass allocation constraints under Mediterranean conditions (Richards et al., 2002; Brunner et al., 2024). Furthermore, a significantly positive correlation was observed between the grain weight of the main and the grain weight of the secondary shoots, reinforcing the additive value of both the main and secondary spikes in determining the final yield (Güngör et al., 2024).

### Grain Physical Traits

Statistical analysis revealed significant differences among barley accessions across multiple quality parameters (Tables 4, 5). The old cultivar Athinaida (H9) demonstrated higher performance in grain firmness (116.92 N), lightness (52.44), and whiteness index (47.80), closely followed by H5 (firmness: 113.90 N; L: 50.24; WI: 47.11). Both accessions exhibited significantly higher values in these key quality traits compared to the rest of the cultivars, underscoring their potential for food and processing applications. However, it should be noted that no biochemical or nutritional quality assessments were conducted, limiting the extent to which these traits can predict actual food processing suitability (Marshall et al., 1986; Berman et al., 1996).

Moreover, the landraces H1, H7, H8, and old cultivar Athinaida (H9) displayed increased bulk and tap densities ( $\geq 0.44 \text{ g cm}^{-3}$  and  $\geq 0.48 \text{ g cm}^{-3}$ , respectively), reflecting compact and uniform grain structures. These characteristics are particularly favorable for industrial processing, packaging efficiency, and storage stability. Regarding colorimetric traits, Athinaida and Triptolemos showed elevated red-green color balance ( $a^*$ ), while Explora-R2 exhibited the lowest ( $b^*$ ) value, indicating reduced yellow pigmentation, an attribute that may influence consumer appeal and visual sorting in processing lines.

Regarding morphometric traits, H1 and H8 stood out for their large average grain diameters (5.89 mm and 5.62 mm, respectively) and long grain lengths (8.41 mm and 8.59 mm), indicating well-developed and full grain. Athinaida (H9) also combined favorable values of roundness (1.37), perimeter (20.43 mm), and density ( $152.81 \text{ g cm}^{-3}$ ), further highlighting its robust grain structure. In contrast, cultivar Explora-R2 presented the highest aspect ratio (2.11) and grain density ( $159.47 \text{ g cm}^{-3}$ ), yet combined these with lower firmness, brightness, and yellowness, traits that may limit its suitability for specific end uses.

**Table 4.** Mean values of quantitative physical and colorimetric grain traits and multiple comparisons among barley accessions using Tukey's test ( $\alpha = 0.05$ )

Treatments	Grain firmness (N)	Volume (cm <sup>3</sup> )	Tap volume (cm <sup>3</sup> )	Bulk density (g cm <sup>-3</sup> )	Tap density (g cm <sup>-3</sup> )	CI	L	a	b	WI
H1	102.91 <sup>b</sup>	101.78	88.22 <sup>b</sup>	0.47 <sup>a</sup>	0.52 <sup>a</sup>	11.66	50.80 <sup>ab</sup>	5.27 <sup>abc</sup>	19.74 <sup>ab</sup>	46.68 <sup>ab</sup>
H2	101.25 <sup>b</sup>	126.89	106.67 <sup>a</sup>	0.39 <sup>ab</sup>	0.47 <sup>a</sup>	15.79	49.72 <sup>ab</sup>	4.16 <sup>abc</sup>	18.09 <sup>ab</sup>	46.39 <sup>ab</sup>
H3	107.32 <sup>b</sup>	94.67	83.56 <sup>bc</sup>	0.36 <sup>ab</sup>	0.41 <sup>ab</sup>	11.36	48.35 <sup>ab</sup>	3.86 <sup>bc</sup>	16.36 <sup>ab</sup>	45.67 <sup>ab</sup>
H4	105.26 <sup>b</sup>	108.89	96.44 <sup>b</sup>	0.39 <sup>ab</sup>	0.43 <sup>a</sup>	10.86	50.79 <sup>ab</sup>	5.32 <sup>abc</sup>	19.56 <sup>a</sup>	46.72 <sup>ab</sup>
H5	113.90 <sup>ab</sup>	95.67	82.22 <sup>bc</sup>	0.42 <sup>ab</sup>	0.47 <sup>a</sup>	11.56	50.24 <sup>ab</sup>	4.03 <sup>abc</sup>	17.41 <sup>ab</sup>	47.11 <sup>a</sup>
H6	100.54 <sup>b</sup>	100.78	85.89 <sup>b</sup>	0.39 <sup>ab</sup>	0.44 <sup>a</sup>	13.10	48.22 <sup>ab</sup>	4.84 <sup>abc</sup>	16.03 <sup>ab</sup>	45.33 <sup>ab</sup>
H7	96.96 <sup>bc</sup>	102.89	90.00 <sup>b</sup>	0.45 <sup>a</sup>	0.50 <sup>a</sup>	10.54	50.31 <sup>ab</sup>	4.45 <sup>abc</sup>	18.68 <sup>ab</sup>	46.70 <sup>ab</sup>
H8	107.85 <sup>ab</sup>	102.89	93.11 <sup>b</sup>	0.45 <sup>a</sup>	0.48 <sup>a</sup>	7.21	49.64 <sup>ab</sup>	2.93 <sup>c</sup>	16.56 <sup>ab</sup>	46.74 <sup>ab</sup>
H9	116.92 <sup>a</sup>	99.33	88.22 <sup>b</sup>	0.44 <sup>a</sup>	0.49 <sup>a</sup>	9.99	52.44 <sup>a</sup>	6.02 <sup>ab</sup>	20.39 <sup>a</sup>	47.80 <sup>ab</sup>
H10	96.69 <sup>bc</sup>	87.78	74.89 <sup>cd</sup>	0.44 <sup>a</sup>	0.49 <sup>a</sup>	12.17	46.91 <sup>ab</sup>	6.79 <sup>ab</sup>	10.98 <sup>bc</sup>	44.12 <sup>ab</sup>
H11	93.99 <sup>c</sup>	76.00	71.33 <sup>cd</sup>	0.51 <sup>a</sup>	0.54 <sup>a</sup>	5.16	49.93 <sup>ab</sup>	5.94 <sup>ab</sup>	19.63 <sup>ab</sup>	45.87 <sup>ab</sup>
H12	91.65 <sup>c</sup>	89.11	78.67 <sup>c</sup>	0.25 <sup>b</sup>	0.29 <sup>b</sup>	11.31	40.98 <sup>b</sup>	6.27 <sup>a</sup>	5.24 <sup>c</sup>	39.55 <sup>b</sup>
Prob > F <sup>1</sup>	*	ns	*	*	*	ns	*	**	*	*
R <sup>2</sup> <sup>2</sup>	0.47	0.25	0.47	0.41	0.52	0.19	0.51	0.67	0.57	0.52
CV% <sup>3</sup>	14.52	26.14	21.75	27.29	21.25	61.93	8.82	27.26	38.09	6.91
G. Mean <sup>4</sup>	102.9	98.88	86.60	0.41	0.46	49.03	42.23	4.98	16.55	45.72

<sup>1</sup> Prob > F: Significance level; \* / \*\* / \*\*\*:  $p < 0.05$  / 0.01 / 0.001; ns: Not significant; <sup>2</sup> R<sup>2</sup>: Coefficient of determination; <sup>3</sup> CV%: Coefficient of variation;

<sup>4</sup> G. Mean: Grand mean; Different letters indicate significant differences based on Tukey's *test*.

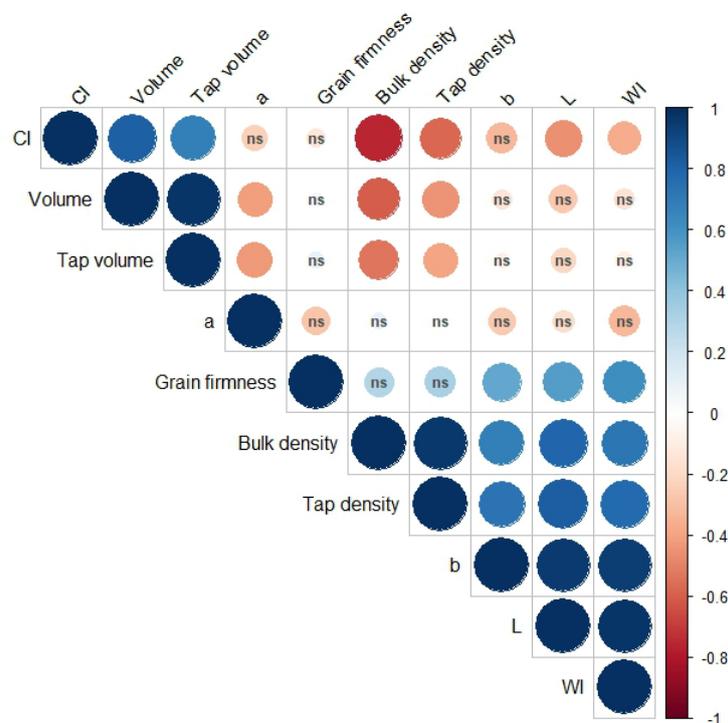
**Table 5.** Mean values of geometric grain traits and multiple comparisons among barley accessions using Tukey's *test* ( $\alpha = 0.05$ )

Treatments	Grain area (mm <sup>2</sup> )	Aspect ratio	Density	Diameter max (mm)	Diameter min (mm)	Diameter ave (mm)	Perimeter (mm)	Roundness	Grain length (mm)	Grain width (mm)
H1	28.17	1.97 <sup>ab</sup>	143.79 <sup>b</sup>	8.38 <sup>a</sup>	4.25	5.89	21.56	1.32 <sup>ab</sup>	8.41 <sup>a</sup>	4.37
H2	27.21	2.08 <sup>ab</sup>	146.93 <sup>b</sup>	8.42 <sup>a</sup>	4.12	5.67	21.25	1.35 <sup>ab</sup>	8.41 <sup>a</sup>	4.17
H3	27.04	1.96 <sup>ab</sup>	148.77 <sup>b</sup>	8.19 <sup>ab</sup>	4.18	5.67	21.24	1.33 <sup>ab</sup>	8.20 <sup>ab</sup>	4.34
H4	27.94	1.91 <sup>ab</sup>	147.09 <sup>b</sup>	8.23 <sup>ab</sup>	4.32	5.78	21.41	1.31 <sup>ab</sup>	8.26 <sup>ab</sup>	4.45
H5	26.85	2.07 <sup>ab</sup>	150.78 <sup>b</sup>	8.49 <sup>a</sup>	4.07	5.61	21.55	1.38 <sup>ab</sup>	8.50 <sup>a</sup>	4.22
H6	26.43	1.93 <sup>ab</sup>	148.75 <sup>b</sup>	7.88 <sup>ab</sup>	4.11	5.55	20.73	1.32 <sup>ab</sup>	7.89 <sup>ab</sup>	4.26
H7	26.45	2.02 <sup>ab</sup>	145.18 <sup>b</sup>	8.16 <sup>ab</sup>	4.06	5.59	20.98	1.33 <sup>ab</sup>	8.17 <sup>ab</sup>	4.2
H8	27.13	2.18 <sup>a</sup>	145.35 <sup>b</sup>	8.58 <sup>a</sup>	3.95	5.62	21.55	1.37 <sup>ab</sup>	8.59 <sup>a</sup>	4.07
H9	24.47	2.11 <sup>ab</sup>	152.81 <sup>ab</sup>	8.17 <sup>ab</sup>	3.89	5.4	20.43	1.37 <sup>ab</sup>	8.19 <sup>ab</sup>	4.01
H10	22.54	1.88 <sup>b</sup>	153.4 <sup>ab</sup>	7.30 <sup>b</sup>	3.88	5.19	18.9	1.27 <sup>b</sup>	7.32 <sup>b</sup>	3.99
H11	23.87	1.85 <sup>b</sup>	147.31 <sup>b</sup>	7.55 <sup>ab</sup>	4.07	5.43	19.63	1.29 <sup>ab</sup>	7.57 <sup>ab</sup>	4.18
H12	23.8	2.11 <sup>ab</sup>	159.47 <sup>a</sup>	8.04 <sup>ab</sup>	3.73	5.24	20.46	1.43 <sup>a</sup>	8.06 <sup>ab</sup>	3.91
Prob > F <sup>1</sup>	ns	**	*	*	ns	*	ns	*	**	ns
R <sup>2</sup> <sup>2</sup>	0.46	0.60	0.69	0.61	0.37	0.43	0.46	0.51	0.61	0.33
CV% <sup>3</sup>	8.99	4.83	2.31	4.31	5.92	4.86	5.11	3.68	4.31	6.47
G. Mean <sup>4</sup>	25.99	2.01	149.13	8.11	4.05	5.55	20.80	1.33	8.13	4.17

<sup>1</sup> Prob > F: Significance level; \* / \*\* / \*\*\*:  $p < 0.05$  / 0.01 / 0.001; ns: Not significant; <sup>2</sup> R<sup>2</sup>: Coefficient of determination; <sup>3</sup> CV%: Coefficient of variation;

<sup>4</sup> G. Mean: Grand mean; Different letters indicate significant differences based on Tukey's *test*.

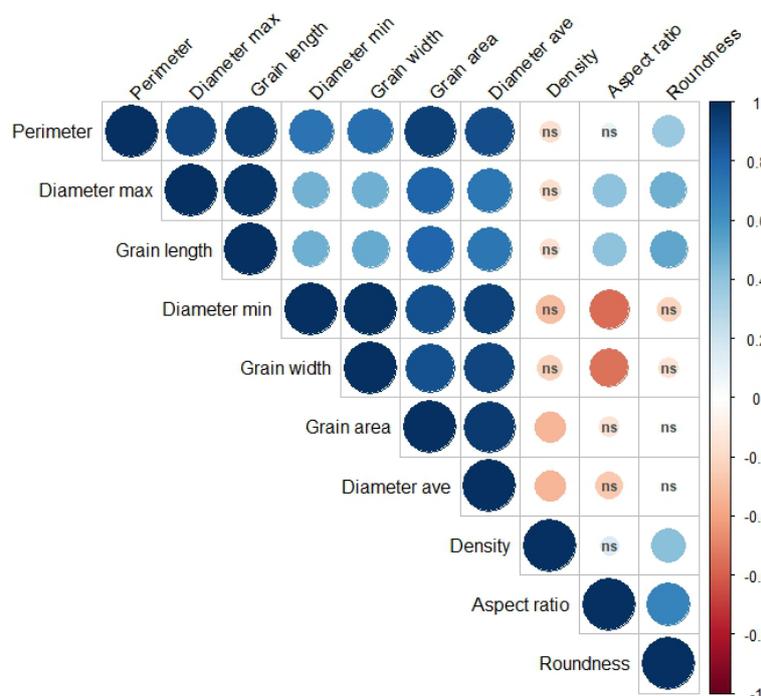
Correlations between various characteristics provided further insights into the relationships among the grain properties (Figs 5, 6). A significantly positive correlation was observed between grain firmness and both bulk density and tap density ( $r = 0.33$  and  $0.35$  respectively), suggesting that firmer grains tend to be denser (Aghajani et al., 2012). This relationship is important for grain processing, as denser grains might affect milling and processing efficiency. Furthermore, a significantly positive correlation between diameter and grain size (both length and width) was found ( $r = 0.73$  and  $0.92$ ). Roundness and perimeter were also positively correlated ( $r = 0.38$ ), indicating that more rounded grains tend to have a larger perimeter, which is expected given that rounder grains are typically more compact and easier to process. Moreover, increased roundness may also be indicative of grain maturity or the absence of morphological defects, as mature grains tend to develop a more symmetrical shape during ripening.



**Figure 5.** Correlation coefficients between physical and colorimetric grain traits of the studied barley accessions ( $n = 36$ ). Circle size shows correlation strength; color indicates direction (red: negative, blue: positive). Non-significant correlations are denoted as ‘ns’.

Color parameters, such as L (lightness) and b (yellow-blue), were significantly positively correlated with the whiteness index (WI) ( $r = 0.97$  and  $0.94$  respectively), meaning that grains with a higher lightness and more yellow hue tend to have better whiteness quality. This has important implications for applications where color and product quality are critical, such as in flour production or other grain-based products (Novaro et al., 2001). However, in composite flour mixtures where color differences are minimal or not visually perceptible, the impact of these color attributes on overall quality may be reduced, highlighting the need for complementary quality parameters in such

contexts. Lastly, there was a negative correlation between grain firmness and the a (red-green) index ( $r = -0.34$ ), suggesting that firmer grains tend to have a lower ‘red’ intensity.



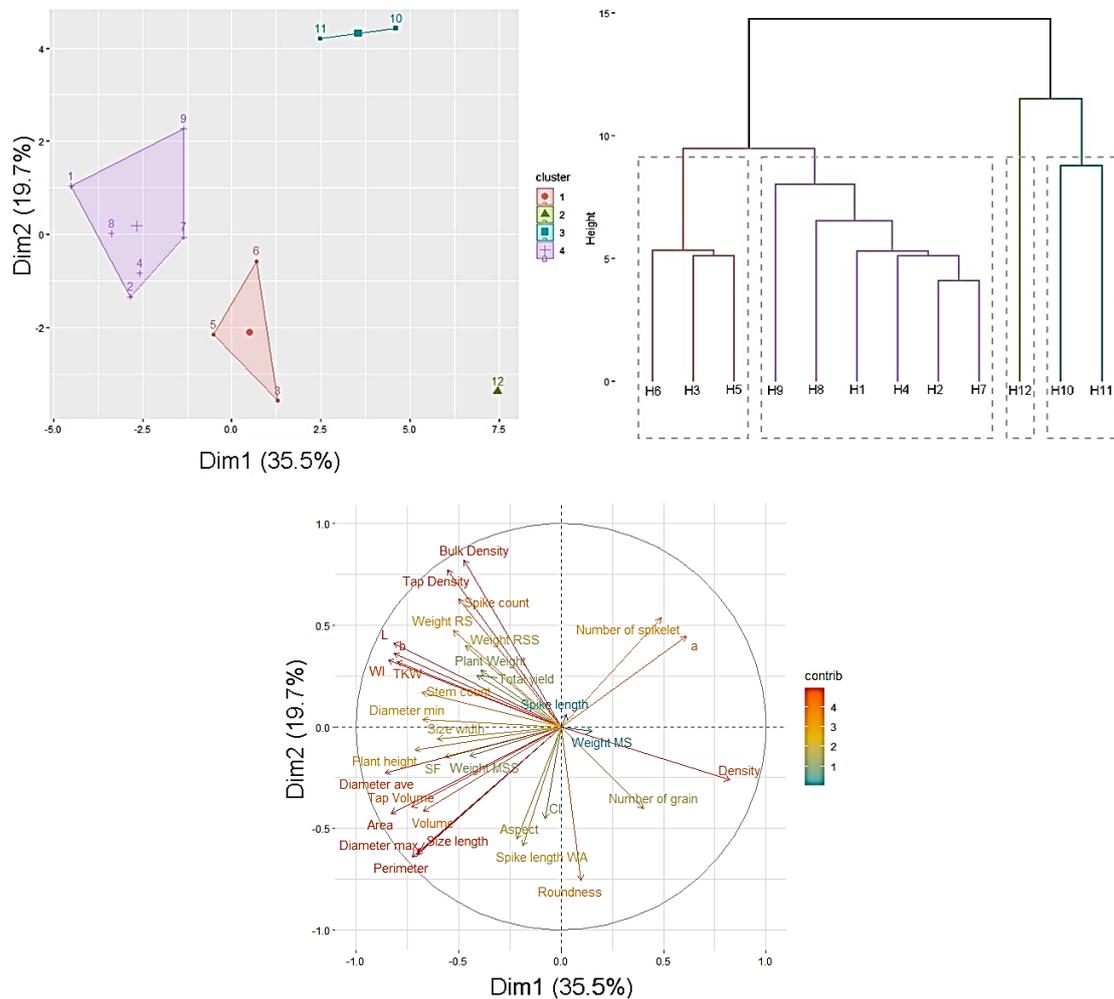
**Figure 6.** Correlation coefficients between geometric grain traits of the studied barley accessions ( $n = 36$ ). Circle size shows correlation strength; color indicates direction (red: negative, blue: positive). Non-significant correlations are denoted as ‘ns’.

Landrace H6 and old cultivar H9 exhibited the highest grain firmness, making them more suitable for industrial processing applications where durability is required (Marshall et al., 1986; Berman et al., 1996). Larger and more spherical grains, as observed in landraces H6 and H8, were associated with better processing quality and higher commercial preference. This is an important finding for grain processing, as larger tend to yield better results in milling and are generally preferred in certain industrial applications (Marshall et al., 1986; Berman et al., 1996; Novaro et al., 2001). Larger grains also typically have higher market value and are more efficient in processing. Moreover, color parameters such as whiteness and lightness, which were higher in accessions H8 and H9, could enhance consumer acceptance in food industries. These differences in color may influence the consumer acceptance of grains in the market, with brighter and whiter grains generally being preferred.

### Grouping of Accessions

Cluster analysis based on agronomic and grain physical traits clearly distinguished the barley accessions according to their geographic origin and breeding status (Fig. 7). The first two principal dimensions explained 55.2% of the total variation (35.5% and 19.7%, respectively), with PC1 mainly associated with grain physical traits (average

diameter, whiteness index, area, thousand kernel weight) and PC2 reflecting variation in bulk density, roundness, spike count, and seed length.



**Figure 7.** Cluster plot, dendrogram, and PCA biplot of barley accessions based on all recorded traits.

Aegean Island landraces formed a distinct cluster, characterized by smaller, harder seeds and shorter plants, traits that likely reflect adaptation to dry and nutrient-poor island environments. Mainland accessions (e.g., H3, H8) and the old cultivar Athinaida (H9) clustered more closely with other landraces, while modern cultivars (Triptolemos, Thessaloniki, Explora-R2) grouped separately, showing high morphological uniformity as a result of targeted breeding for productivity and agronomic stability. Notably, some traditional accessions (e.g., H8, H9) performed as well as or better than modern cultivars in several agronomic traits, highlighting their potential for use in breeding programs. Despite their morphological diversity, landraces shared structural traits such as spike architecture and seed hardness, indicating parallel adaptation to traditional, low-input farming systems, in agreement with findings reported for other cereal landraces (Korpetis et al., 2023).

## CONCLUSIONS

This study highlighted the significant phenotypic variability among cultivar and landrace barley accessions under Mediterranean conditions. Agromorphological trait analyses revealed strong genotype effects and significant accession  $\times$  year interactions, while grainphysical traits displayed distinct genotypic differences. Landraces, particularly those from the Aegean islands, exhibited unique morphological and physical profiles, such as smaller but firmer grains and reduced plant height, traits that may reflect long-term adaptation to arid and low-input environments. Cluster analysis effectively grouped accessions according to geographic origin and breeding status, highlighting the structural divergence between landraces and modern cultivars. Notably, several accessions (e.g., H1, H4, H8, H9) demonstrated agronomic performance comparable to or exceeding that of modern cultivars, showing promising levels of yield stability and adaptive potential under Mediterranean field conditions. These traits may indicate a degree of resilience; however, further physiological studies and evaluation under targeted abiotic stress conditions are required to substantiate this adaptive potential. While the findings offer valuable insights into the phenotypic diversity and agronomic potential of Greek barley landraces, their broader validation would benefit from additional testing across environments and under defined stress conditions. Such efforts could help consolidate the present conclusions and further support their relevance for breeding and adaptation.

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